

Effect of Sugar Concentrations on Bacterial Cellulose Production as Cellulose Membrane in Mixture Liquid Medium and Material Properties Analysis

Faridah¹, Selvie Diana², Helmi³, M. Sami⁴, Mudliana⁵

^{1,2,3,4,5}Faculty of Chemical Engineering, State of Polytechnic Lhokseumawe
Lhokseumawe, 24301, Aceh, Indonesia
Email: ida4699@yahoo.com

Abstract— Cellulose membrane is bacterial cellulose which produced from the fermentation process by *Acetobacter xylinum*. Cellulose membrane was performed in mixture liquid medium by using coconut water and sugar palm juice as the base medium. In this study, different present sugar concentrations including 0%, 7.5% and 10% were added into fermentation medium to enhance bacterial cellulose production. By adding three different sugar concentrations, the bacterial cellulose from different sugar concentration was made into cellulose membrane. Cellulose membrane were tested for physical properties of cellulose membrane, such as the degree of crystallinity by x-ray diffraction (XRD), water content and thermal decomposition behaviour by TGA, scanning electron microscopy analysis (SEM), tensile strength, FTIR spectra of cellulose membrane. The better cellulose membrane was reported from the addition of 7.5% sugar for all physical properties material.

Keywords— Sugar; *Acetobacter xylinum*; medium; cellulose membrane.

I. INTRODUCTION

Bacterial cellulose can be produced from fermentation process by *Acetobacter xylinum* at the liquid medium which contains carbon source such as coconut water [1]. The main content of bacterial cellulose is cellulose which has chemical purity and crystallinity higher than plant cellulose [2]. Even though plant cellulose and bacterial cellulose have the same chemical structure³, bacterial cellulose has ultra-fine network structure, high biodegradable and high mechanical strength [1] and bacterial cellulose also shows unique properties, including higher degree crystallinity, higher water retention ability, higher tensile strength and better mold ability [4], [5]. Hence, bacterial cellulose can be applied in many industrial applications such as the pharmaceutical industry [6], food industry [7], wound care productions [8] material constructions [9] and pulp and paper industry [10], [11]. Bacterial cellulose can be used as membrane dialysis [12]. In the beginning, membrane was mostly made from polymer synthetic material. Membrane from bacterial cellulose have similar structur with membrane from polymer synthectic material. Thus, cellulose membrane can be applied as membrane. Cellulose membrane is semi-permeable thin layer

and can be used to separate the two components by restraining certain components pass through the pores. Moreover, membrane cellulose also has some advantages in the separation process; the separation can be operated in continuous way and does not need too much energy [13]. The first study shows that membrane cellulose from bacterial cellulose has high mechanical strength and highly hydrophilic holding water [14]. Therefore bacterial cellulose can be performed separation membrane.

In this study, membrane cellulose was produced by *Acetobacter xylinum* in mixture liquid medium by using coconut water and sugar palm juice as the base medium. The various sugar concentrations including 0%, 7.5% and 10% were added into fermentation medium to enhance bacterial cellulose production. By adding three different sugar concentrations, the bacterial cellulose from different sugar concentration was made into membrane cellulose. In general, there are many kind of sugar which used as carbon source to produce bacterial cellulose as membrane cellulose. Glycerol, glucose, fructose, inositol, saccharides and beet molasses has been used as a carbon source [15-17]. They have a relatively higher degree of polymerization and no remarkable differences in crystallinity index of cellulose [16-18]. Furthermore, the difference concentrations of sugar as a carbon source were also used for bacterial cellulose as cellulose membrane. Sugar is one of a carbon source to produce bacterial cellulose by *Acetobacter xylinum*.

The goal of this study was to evaluate effects of the difference sugar concentrations to produce membrane cellulose by *Acetobacter xylinum* and analyse material properties and this study have some objectives including; (1) to evaluate the yield of bacterial cellulose as membrane cellulose in mixture liquid medium (coconut water and sugar palm juice) with various concentrations of sugar; (2) to dertemine the degree of crystallinity by x-ray diffraction (XRD); (3) Water content and thermal decomposition behaviour by TGA; (4) scanning electron microscopy analysis (SEM) for determining the structure of membrane cellulose; (5) Universal Tensile Machine for determining tensile strength of membrane cellulose; (6) FTIR spectra of cellulose membrane.

II. MATERIALS AND METHODS

A. Culture Conditions

Acetobacter xylinum culture obtained from Biotechnology laboratory of state Polytechnic lhokseumawe, Aceh, Indonesia. The cultures were incubated statically at room temperature in coconut water as culture medium. Three hundred mL of coconut water mixed with 8 g glucose, 2 g ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$) and 0.5 mL glacial acetic acid (CH_3COOH). All media were sterilized at 121 °C for 15 min. This medium was boiled for 10 min. *Acetobacter xylinum* grown on this medium. *Acetobacter xylinum* was activated in coconut water and inoculated statically at room temperature around 30 °C for 7 days.

B. Bacterial Cellulose production

All chemical were used to produce bacterial cellulose as nutrient. The culture medium was added some nutrients for bacterial cellulose production including sugar, acetic glacial, ammonium sulphate. The culture medium of bacterial cellulose production consists of 50% of coconut juice and 50% of sugar palm juice (*arenga pinnata* juice). Three hundreds of the mixture culture medium were added into 1 mL beaker glass and added 8 g of sugar, 2 g of ammonium sulphate, and 0.5 mL of acetic acid glacial and then stirring until homogeneity. This medium was boiled and was cooled at room temperature. Starter of *Acetobacter xylinum* gently was added in culture medium and fermented at 6-7 days in static batch to produce bacterial cellulose [19] and weighed.

C. Preparation of cellulose membrane

Bacterial cellulose was washed with distilled water until the pH neutral and pre-treated with 2% NaOH for 24 hours. After soaking, the membranes were neutralized with distilled water and were be pressed by hydraulic press to become film of cellulose membrane. Then, film of cellulose membrane boiled with distilled water for 10 minute, thoroughly dried at 90°C for overnight and weighed. These procedures were done for each difference sugar concentration.

D. X-ray diffraction (XRD)

The x-ray diffraction (XRD 7000 Shimadzu) was used to determine the crystallinity of cellulose membrane; the x-ray diffraction (XRD) patterns of samples were collected on a Scanting PAD V theta-2theta diffractometer using a copper X-ray source. Scans were collected at 2 deg/min and 10 to 70 degree 2 . Samples of cellulose membrane were lyophilized overnight at 0,133 mbar fist by using a lab-scale freeze-dryer (Model Free zoom 2,5 L, Labconco Co., Kansas, MO) and pressed into a thin and flat layer for analysis. Materials Data, Inc., Livernore, (CA) was used to process diffraction pattern and calculate the crystallinity of cellulose membrane [20].

E. Thermogravimetric Analysis (TGA)

The dynamic weight loss tests were determined on a thermo gravimetric analyser (TGA) machine (model Shimadzu DTG 60). For water content determination of cellulose membrane, all sample tests were conducted in a N_2

purge (40 ml/min) over a temperature range 30-650°C at an increase rate of 10°C/min. Cellulose membrane samples were placed on the absorbent wipers to eliminate excess water and thermal decomposition test, cellulose membrane samples were dried to 80°C and analyzed then conducted in a N_2 purge (15 ml/min) over a temperature range 80-600°C at an increase rate of 20°C/min [20].

F. Microscopic of cellulose membrane by using Scanning Electron Microscope (SEM)

Cellulose membrane was fixed and dehydrated on SEM merk JEOL JSM-6510LA . Cellulose membrane were cut with diamond and coated with gold. SEM JEOL JSM-6510LA field emission scanning electron microscope at 10 or 15 kV was used for sample examination. The picture were captured both top view and side view with 500x magnification [21].

G. Tensile Testing

Specimens from cellulose membrane were mounted into 5 N load cell of Universal Tensile Machine. Cellulose membrane for specimen were cut base on ASTM D-882 standards [22]. One load cell statically fixed with the tensile machine frame and second one fixed with the moveable part of the machine. Specimens were strained at displacement rate of 1 mm min⁻¹. The specimen samples were deformed under controlled room condition at temperature of 23±1 °C and humidity of 50±5% [23].

H. FTIR of cellulose membrane

FTIR spectra were prepared on IR-Prestige-21 Shimadzu spectrometer. The samples could be analysed without dilution and samples spectra were acquired in rapid scan mode with a 1 kHz modulation frequency and by averaging scan at 4 cm⁻¹ resolution [11].

III. RESULTS AND DISCUSSION

A. Comparison of Bacterial Cellulose Production and Cellulose Membrane with addition of different sugar concentrations

Sugar was added in mixture culture medium to produce bacterial cellulose as cellulose membrane. The various sugar concentrations including 0%, 7.5% and 10% (% weight) was added in mixture liquid medium. The mixture culture medium consist of coconut water and sugar palm juice (*Arenga pinnata juice*) the base medium for producing cellulose membrane. This study found that adding 7,5% sugar obtained the optimum yields for all medium for bacterial cellulose production (fig.1). It is because sugar has 99% sucrose which the major component of sugar. The carbon source in mixture liquid medium was replaced with different sugar concentrations (fig. 1). Addition of sugar in culture medium was influenced the yields of bacterial cellulose and membrane cellulose. The bacterial cellulose yields increased at 7,5% sugar and decreased gradually at 10% sugar. However sugar levels greater than 5% have been reported to have no

advantage to increase the efficiency of cellulose production [24] and 10 % sucrose concentration was found to maximize the thickness of bacterial cellulose production [1]. Sugar also can be found in medium which consists of sucrose as a source carbohydrate. Sugar in medium can also influence bacterial cellulose. Where sugar palm juice contains 11.28% carbohydrate [25] and coconut juice contains 7.29% carbohydrate. On the other hand, the addition of 7.5% sugar in the culture medium is the optimum for the growth of *Acetobacter xylinum* to convert glucose into cellulose and the addition of sugar above 7.5% will give the dehydration in cells *Acetobacter xylinum*, it can produce the lower cellulose production.

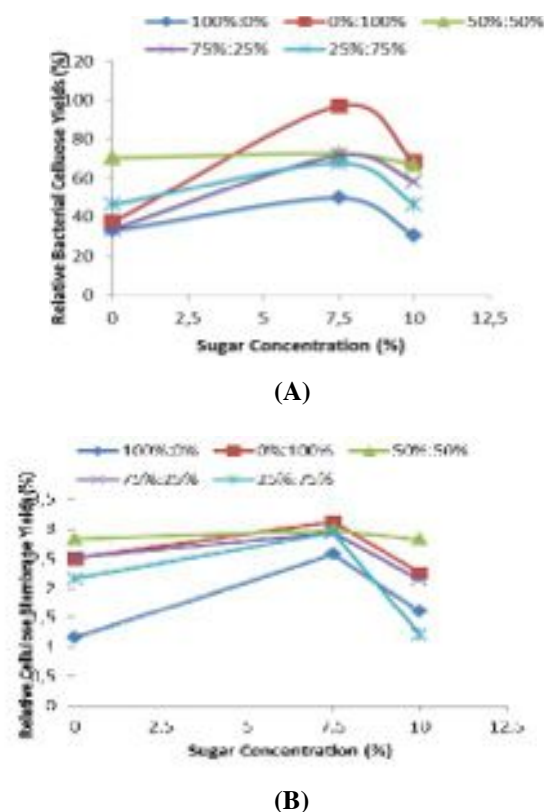


Figure 1. Effect of sugar concentration on bacterial cellulose production (A) and cellulose membrane production (B) in mixture liquid medium by *Acetobacter xylinum* (the ratio of the culture medium consists of sugar palm juice (*arenga pinnata* juice) and coconut water to produce edible film including \blacklozenge = 100%:0%, \blacksquare = 0%:100%, \blacktriangle = 50%:50%, \times = 75%:25%, \ast = 25%:75%.

Sugar concentration in the culture medium was found to positively effect on the bacterial cellulose production and cellulose membrane production. Recently, this study found that 7.5% sugar addition in 100% coconut juice was the highest bacterial cellulose and cellulose membrane production and the lowest bacterial cellulose was found in 100% sugar palm juice. This is due to sugar palm juice is susceptible to fermentation process for yeast and bacteria including

Saccharomyces and *Acetobacter acetic* [26]. Sucrose in sugar palm juice breakdown into glucose and fructose and then produces alcohol and vinegar. Sugar as a carbon source is the energy source of bacterial with the formation of cellulose bacterial cells [27]. Although bacterial cellulose yields is equivalent to the cellulose membrane yields producing in the different culture medium (Fig. 1). Effects of sugar concentration on the bacterial cellulose as cellulose membrane production were respectively analysed physical property of cellulose membrane.

B. Cellulose membrane structure and physical properties

In this study, different sugar concentrations including 0%, 7.5% and 10% were examined for cellulose membrane production from bacterial cellulose by *Acetobacter xylinum*. Sugar was exhibited as a carbon source to produce bacterial cellulose which has high hydrophobicity and has significant water holding capacity [14]. Sugar as a carbon source was used in the fermentation process. It is form carbohydrate compounds that are classified monosaccharides and disaccharides. Basically, the addition of sugar concentration can affect the texture of cellulose membrane.

To see the structures of cellulose membrane, microscopic study on micro-structures of cellulose membrane by using a SEM shows that the top views of cellulose membrane analysed. Figure 2 showed that the cellulose membrane with 7.5% sugar addition was smoother than cellulose membrane from the other cellulose membrane. While 0% sugar addition showed that the surface of cellulose membrane was rougher than 10% sugar and 7.5% addition. It was found that the addition of sugar affected the cellulose fibrils arrangement at microscopic level and it could influence structure of cellulose membrane.

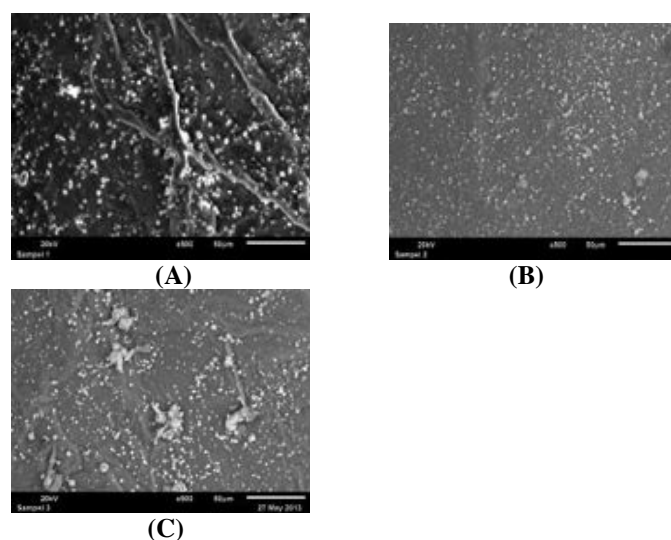


Figure 2. Scanning electron micrographs show the morphology of cellulose membrane films (A=0% sugar addition, B=7.5% sugar addition, C=10% sugar addition) as a function of sugar concentration addition in the mixture liquid medium (50% sugar palm juice and 50% coconut water)

XRD patterns were obtained from cellulose membrane samples. They have no high difference in crystal structure for three cellulose membrane productions from 0% sugar, 7.5% and 10% sugar addition. It may be that they have the smaller crystallize sizes of micro fibrils resulted. This study found three main characteristic peaks standards for crystal planes $\langle -110 \rangle$, $\langle 110 \rangle$, and $\langle 200 \rangle$ which was compared with cellulose standards (figure 3). The crystal structure is mainly cellulose 1- β form [20], [28]. Crystallinity of the membrane will affect its performance; the degree of crystallinity of the membrane was determined. Cellulose membrane is a semi-crystalline polymer as seen XRD pattern that showed a peak between 10° and 25° at 2θ .

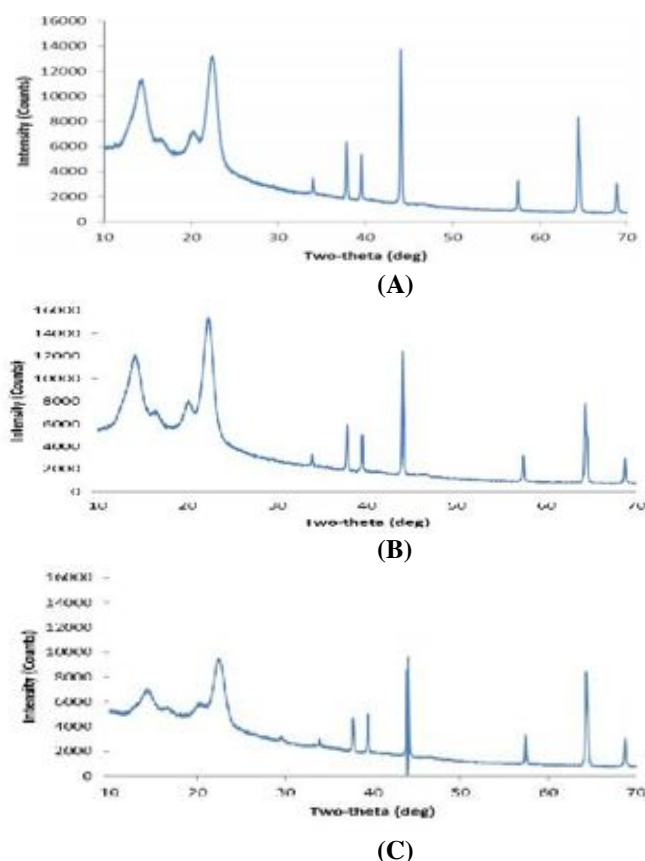


Figure 3. X-ray diffraction patterns of cellulose membrane produced by *Acetobacter xylinum* in the mixture liquid medium (50% sugar palm juice and 50% coconut water) with the addition of 0% sugar (A), 7.5% sugar (B) and 10% sugar (C).

FTIR analysis was conducted to determine the pattern of changes in spectral characterization and interaction groups existing functioning of cellulose membrane (fig. 4). From cellulose membrane samples, FTIR spectra were demonstrated for three cellulose membrane from different sugar concentration. They have no high difference carboxyl group, were found OH functional groups detected at wave numbers 3000 to 3556.74 cm^{-1} . The CH stretching vibration can be shown by the peak at wave number 2850 cm^{-1} and 2950 cm^{-1} .

Cellulose is composed of glucose units. Glucose is not absolutely in a state of cyclic but glucose can also be stabilized in the form of an open chain in the Fischer projection. This is reinforced by the presence of C=O group at wave number 1650 cm^{-1} . Addition of different sugar concentration did not change the functional group; it is just different wave absorption intensity.

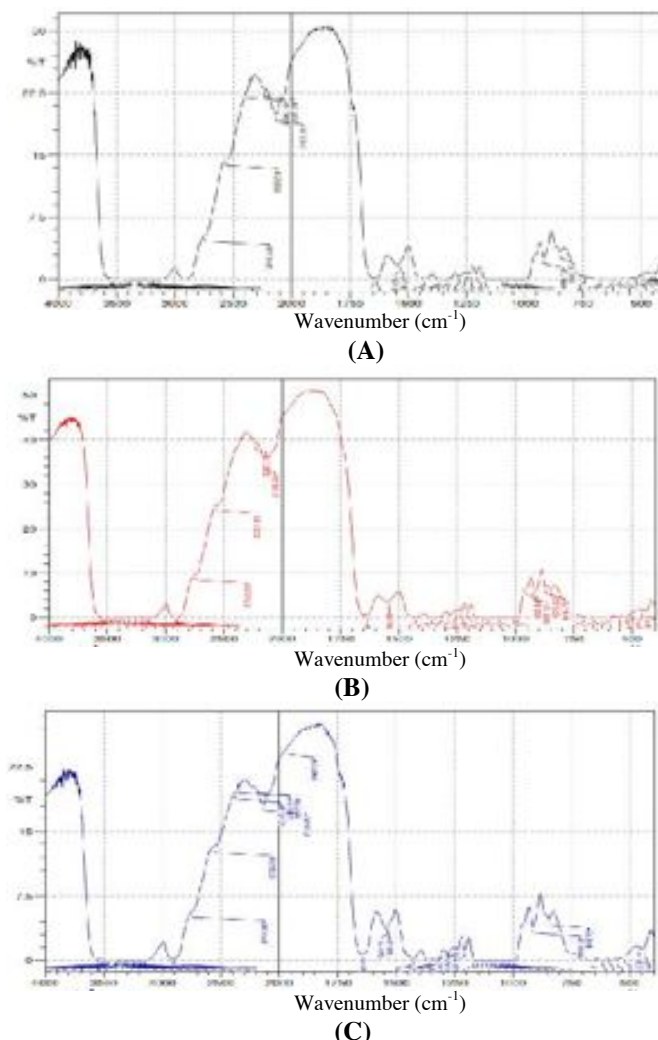


Figure 4. FTIR spectra: Cellulose Membrane with 0% sugar addition; (B) Cellulose with 7.5% sugar addition and (C) 10% sugar addition in the mixture liquid medium by *Acetobacter xylinum*

C. Thermogravimetric Analysis

Thermal decomposition behaviour of cellulose membrane samples was obtained. Figure 5 shows stability thermal of cellulose membrane which was determined by TGA analysis. From different cellulose membrane samples, they have no different results. Where figure 5 showed two steps for weight loss of cellulose membrane, it is indicating the possibility of two types of decomposition [20]. The weight loss at lower temperature

ranging from 300°C to 360 °C has the same pattern which illustrated Yang and Chen from 200°C to 360°C. It was indicated the release of water and may be the removal of small molecular fragments such as hydroxyl and methyl hydroxyl groups [29]. The second weight loss ranging from 360°C to 600°C showed the degradation of polymeric chains and the six-member cyclic structure, pyran [20]. The Thermal stability of cellulose membrane have been reported which the thermal stability of cellulose membrane is affected by some structure parameter s of this cellulose membrane.

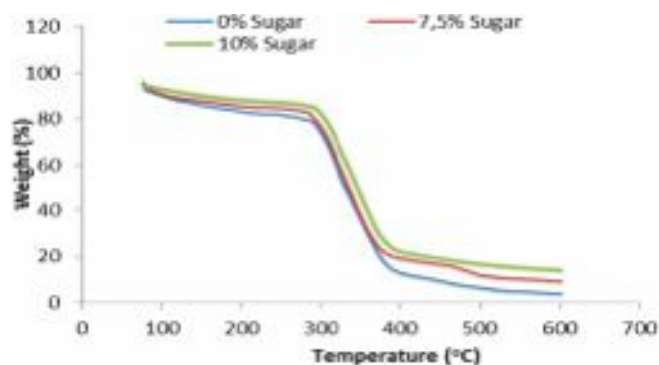


Figure 5. The TGA curves of cellulose membrane produced on the mixture liquid medium with the different sugar concentrations by *Acetobacter xylinum*

D.Mechanical Properties

The quality of cellulose membrane depends on the tensile strength and elongation of cellulose membrane. Tensile strength is one to measure the mechanical properties the power of cellulose membrane. The tensile strength of cellulose membrane was influenced by adding sugar in the culture medium. Figure 6 (B-C) can be seen that the addition of 7.5% sugar concentration into the liquid medium increased the tensile strength of cellulose membrane and also more elastic. Hence, the tensile strength of cellulose membrane gradually decreases at the addition of 10% sugar. It is influenced by the growth of bacteria *Acetobacter xylinum*. On the addition sugar concentration, above 7.5 % could cause plasmolysis in *Acetobacter xylinum* cells, thus reduce the formation of cellulose and it will give a low tensile strength.

Elongation of cellulose membrane is also very effect on the quality of cellulose membrane (Figure 6A). Precent elongation is inversely straight with a tensile strength of the film. Precent elongation is also affected by content of cellulose in addition sugar concentration in the liquid medium for cellulose membrane. The higher elongation was found in the addition of 7.5% sugar and become lower in the addition of 10% sugar in the culture medium. Due to sugar was also influenced by growth *Acetobacter xylinum* bacteria. As for the addition of sugar concentrations above 7.5 % had caused occurrence of plasmolysis in cells *Acetobacter xylinum*, thus lowering formation of cellulose and cause precent extension becomes maximum.

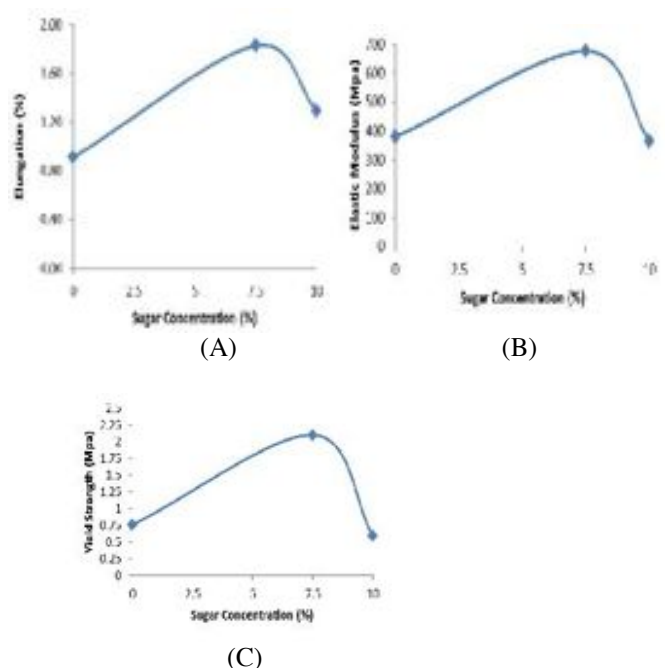


Figure 6. Tensile test of cellulose membrane. (A) Elongation; (B) Elastic Modulus; (C) Yield Strength

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